

PROTOCOL FOR TRICHOMONAS DIAGNOSIS IN CATTLE FOR UTAH - AUGUST 2000

DIAGNOSIS OF TRICHOMONAS:

Diagnosis of trichomoniasis is made when trichomonad organisms are observed in the smegma or preputial flush samples of bulls, or the uterine/vaginal fluid of cows. The organisms may be observed by direct microscopic examination of the fresh samples or by examination of culture media inoculated with infected material.

SAMPLE COLLECTION:

1. The preferred sample is from the **glans penis**. This can be obtained by using a sterile insemination pipette and performing a vigorous back and forth scraping motion along the glans while applying negative pressure with an attached 10 ml syringe. A separate pipette and syringe should be used for each animal.
2. A current official Trich tag should be placed in the right ear of each bull at the time of sample collection.
3. The preferred sample from the female is the cervical mucous or uterine secretions. These samples can be collected by applying negative pressure with a syringe attached to a sterile insemination pipette, while the pipette is positioned within the open cervix or positioned to collect fluid from the vaginal floor.

MEDIA INOCULATION:

1. Modified Diamond's Media (MDM) (Tube Media): Saline or Lactated Ringer's Solution may be used to flush the smegma or mucous from the AI pipette and then it is poured carefully into the top of the tube media. Place 1-2 ml of either solution into a small test tube prior to collection of samples. After collecting a sample in the pipette, draw the solution from the test tube up and down in the AI pipette to clean it out or, draw the 1-2 ml of either solution into the syringe prior to scraping and then flush the pipette with it and flush into a test tube. Cap and label the tube to identify the animal sampled.

These samples should be transferred onto the MDM (tube media) within 1-2 hours after collection (maximum of 6 hrs). They can be hand-carried to the lab and transferred there, IF that can be done within 6 hours. Keep the samples out of direct sunlight and away from excessive heat or cold (maintain at 65-75 degrees F).

To transfer the sample to media, tap the sample tube to mix the smegma and solution, uncap both tubes, tip both at an angle and pour the sample tube gently down the inside of the MDM tube. Cap and identify. Keep at 65-75 degrees F.

You may carefully inoculate the sample directly into the upper tubes of Modified Diamond's Semisolid Media. Do Not put the AI pipette way down into the media. Do NOT squirt the pipette contents into the tube. Draw 1-2 ml of media into the syringe prior to scraping. Use that media to carefully flush the smegma from the pipette and carefully dribble it onto the top of the tube media, or after scraping draw the top 1-2 ml of media up into the AI pipette to flush the pipette. But this must be carefully layered back onto the top of the media.

This care in flushing and layering is very important since the trichomonads need to migrate to the bottom away from other contaminating organisms. Mixing the media or inverting the tube will inhibit growth of trich. Identify and cap the tubes. Avoid shaking the tubes and do NOT invert them.

2. InPouch TF: Inoculate the sample into the small upper chamber of the pouch and fold the wire strips down at least two folds to seal the upper chamber.
 - a. If a direct microscopic examination is to be made at the time of collection, position the upper chamber into the viewing apparatus and close the frame over the raised platform and the pouch. Observe microscopically using the low power (10 X objective) for presence of motile trichomonads. Use the higher power (40 X objective), if necessary, for confirmation. **(If no direct microscopic examination is to be performed at this time, proceed directly to step b).**
 - b. Incorporate the contents of the upper chamber into the lower in the following manner: Squeeze or roll up the pouch from the bottom until the culture media in the lower chamber ruptures the breakable seal between the two chambers and washes into the upper chamber. Manipulate the liquid around slightly to intermix the inoculum and media, and then squeeze or squeegee the liquid back into the lower chamber. Carefully express air bubbles out of the lower chamber. Keep the packet upright at all times. (These procedures help to maintain the anaerobic quality of the media but cause minimal disruption of the lower chamber.) Fold the wire strips the rest of the way down (like a whirl-pak) to seal the lower chamber.

SHIPPING & HANDLING:

NOTE: *If tube media is used, it must be hand delivered to the laboratory. It CANNOT be sent by commercial carrier. The tubes must not be inverted.

***In-Pouch TF media can be transported by commercial carrier, but this must be by overnight express/one-day delivery. Samples should not be put in the lower chamber until it arrives at the lab.**

***Be sure the lab will be open to receive samples.**

The shipping and handling of the inoculated media samples is one of the most critical steps in Trichomoniasis diagnosis. It is important to arrange shipping so that the samples arrive at the Laboratory or Clinic that will perform the testing within 30 hours of

collection. Only those samples which are received at a certified diagnostic facility within 30 hours from time of collection will be considered as conforming to requirements for a valid test.

1. It is important to insure that the inoculated media does not get overly shaken or agitated during handling or transport back to the clinic or laboratory. The MDM tubes must **NOT** be inverted.
2. The inoculated media should be kept at 65° F to 75° F until it is incubated. It is especially important to avoid overheating or freezing. Ship the inoculated pouches in insulated containers (**no ice**) that will protect the samples from extreme temperatures. Trichomonads are very susceptible to either freezing or overheating.

CULTURING PROCEDURES:

1. Modified Diamond s Media: Specimens hand delivered and already inoculated into the Modified Diamond s Media may need to be examined microscopically upon arrival (**See Exam Procedures below**) and then put into the incubator. Specimens that were collected that same day and brought in are put directly into the incubator. The tubes are incubated vertically (upright) at 37° C and examined until positive growth and confirmation occurs or until they have remained negative for 4 days. (**See Exam Procedures below**). Optimal growth usually occurs around day 1-2. Specimens remaining negative after 96 hours are reported as negative.
2. InPouch TF Media: Specimens arriving in the InPouch TF pouch by shipping may need to be examined microscopically upon arrival (**See Exam Procedures below**) and then put into the incubator. Specimens that were collected that same day and brought in are put directly into the incubator. The pouches are incubated vertically (upright) at 37° C and examined until positive growth and confirmation occurs or until they have remained negative for 96 hrs. (**See Exam Procedures below**). Optimal growth usually occurs around day 1-2. Specimens remaining negative for 96 hours are reported as negative.

EXAMINATION PROCEDURES:

1. Modified Diamond s Media: A small portion of the media is removed from near the bottom of the tube ($\frac{1}{4}$) with a long (9"), sterile pasteur pipette and a drop is placed on a clean glass slide. The slide is placed on the microscope under a 10X objective (100 power) and examined for typical motile organisms. Several samples can be placed on the same slide, but use care to keep them identified as to source. It is better to not use a coverslip. Be sure to use a new pipette for each tube.
2. InPouch TF Media: For microscopic examination, place the raised platform of the open viewing frame on the bottom of the lower chamber of the pouch. Close and lock the frame

over the pouch. (Trichomonads generally will first be found slightly above the bottom border of the chamber). The pouch and frame are then placed on the microscope under a 10x objective (100 power) and examined for typical motile organisms. Focus the microscope on the crystals or debris present in the bottom of the pouch media (**See Interpretation of Results below**).

- a. The day you receive the samples is Day 0. If samples have been in shipment for more than a few hours, it is suggested that you examine the samples the day they arrive. Incubate the samples vertically (upright) at 37°C (98 - 99°F). Samples should be closely examined on days 2 and 4. The results for that day's reading are recorded on the Official Trichomonas Test and Report Form. Record the date read at the top of the column above Readings, number 1" or number 2". Then record the results for each sample in the column for that day's reading. The final results are recorded for day 4, or earlier for those samples on the test form that have already been found positive. (**Note: If you have positives on the first reading, you may want to call the owner and report the positives prior to Day 4**).
- b. Upon completion of the day's 4 testing, the Laboratory performing the test fills out the summary information, records its name and address, and the Laboratory Supervisor or principal technician signs the forms. The forms are then forwarded according to the distribution at the bottom of the form. The forms should be completed in their entirety, since they are a legal document.
- c. As occasionally the trichomonads may progress through a rapid growth (log) phase and die off very quickly, it is suggested (but not required) that the samples also be given a brief cursory look on days 1, 3 and 5 to check for trichomonads.

Interpretation of results:

POSITIVE: A sample is considered positive when viable, motile trichomonad organisms are observed either upon direct microscopic examination of the sample when collected, or in the culture media on any of the reading days.

NEGATIVE: A sample is considered negative when **no** viable, motile trichomonads are observed in the culture media during any of the reading days **and** after 4 day's incubation has been completed. (**NOTE: If no viable trichomonads are seen upon direct microscopic exam when the sample is collected, that is common. Continue on with the culturing of the sample anyway. A sample must be cultured for the full 4 days before it can be called negative. Some strains do not appear until later in the culture period.**

RE-TEST: When the trichomonas organism dies, it immediately loses its motility. Its morphology, however, will degenerate more slowly. With a rapidly growing or heavily inoculated sample, the trichomonad organisms can sometimes overgrow

the media and die off within a 36 - 48 hour time period. That is the reason for the every-other-day reading schedule; you should catch the organisms sometime during their growth phase. However, if you encounter a culture sample with abundant non-motile organisms of typical trichomonad size, mark the test chart as RE-TEST for that sample and request that a second specimen be submitted from that animal as soon as possible. (The trichomonad organisms become quite large and round in an old culture.)

(Reasoning: Some yeasts and spores will be of similar size and morphology to a dead [non-motile] trichomonad. Thus rather than incorrectly calling the animal Positive , a second sample should be immediately requested and read every day [in case it is a very rapidly-growing trichomonad] to observe if actual viable, motile trichomonad organisms are present. If no viable, motile trichomonad organisms are found upon the re-culture, the animal is called negative).

SAMPLE DISPOSAL:

1. In accordance with the EPA and OSHA requirements for disposal of biological wastes, all trichomonas samples should be inactivated before disposal. This is best accomplished by autoclaving the tubes or pouches prior to discarding. If an autoclave is not available or if autoclaving is not practical, inactivate the tubes by adding Clorox, Nolvasan or some other disinfectant to the tubes or pouches and shaking vigorously prior to disposal.
2. All of the tubes or pouches should be discarded at the end of the 4-day incubation period and **all** should be inactivated regardless of whether the final results were positive or negative.

Media:

1. Modified Diamond s Media: The Modified Diamond s Media is a semi-solid media preparation that is specially prepared at the U.S.U. Animal Disease Diagnostic Laboratories (Logan, 435-797-1895 and Provo, 801-373-6383). The price is approximately \$3.00/tube. Please check with the lab of your choice for the current price.

(NOTE: Generally, maximum survival of the incubated trichomoniasis organisms in the currently used Modified Diamond s Media is about 10 days.)

2. InPouch TF Pouch: The InPouch TF pouch is a commercially prepared and packaged proprietary media. The pouches are available from Bio-Med Diagnostics Inc., 1430 Knoll Cir # 101 in San Jose, CA 95112-4608, Phone: 408-451-0400. The current price is \$3.90 per pouch (plus shipping & handling).

(Note: Generally, maximum survival of the incubated trichomonas organisms in the currently used InPouch TF pouch is about 10 - 12 days.)

SPECIAL NOTE: The above-listed media from the above-listed sources are currently the only officially recognized media for the culturing of bovine trichomoniasis organisms in the State of Utah. Other media from other sources are not currently recognized as official, and other testing methods (i.e. DNA probe, etc.) are not currently recognized as official.

POSITIVE BULLS (from Regulation on Trichomoniasis)

All bulls which test positive to Trichomoniasis must be sent by direct movement within 14 days, to: 1) slaughter at an approved slaughter facility, or 2) to a Qualified Feedlot for finish feeding and slaughter, or 3) to an approved auction market for sale to one of the above facilities. Such bulls must move only when accompanied by a VS 1-27 Form issued by the testing veterinarian or other regulatory official. Positive bulls entering a Qualified feedlot, or Approved Auction Market shall be identified with a lazy V brand on the left side of the tail, indicating that the bull is infected with the venereal disease, Trichomoniasis.